REGENERATION OF THE LIVER IN RATS WITH SARCOMA M1

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A sharp increase in the number of mitoses and binuclear cells and in the size of the nuclei of the hepatocytes is observed in the liver of rats with subcutaneously transplanted sarcoma M1. If a perforating wound of the liver is inflicted in animals with tumors, the zone of necrosis is larger and the rate of formation and maturation of the granulation tissue is slower, but division of the hepatocytes takes place more intensively than in the control. Regeneration of the liver in animals with tumors after resection by the method of Higgins and Anderson takes place rather more rapidly than in the control for the first 8 days, but subsequently the rate becomes slower.

The presence of a focus of malignant growth in the body induces functional and morphological changes in several organs including the liver [1-3, 5]. Insufficient attention has so far been paid to the study of regenerative processes in the liver of animals with tumors [7].

EXPERIMENTAL METHOD

Experiments were carried out on 330 noninbred albino rats weighing 120-150 g and subdivided into 3 groups. Some of the animals had no tumors, while the rest had a sarcoma M1 transplanted subcutaneously on the right side of the abdomen. The state of the liver at various times after transplantation of the tumor was studied in 60 animals of group 1; perforating wounds of the central lobe of the liver were inflicted with a metal rod 2 mm thick on 80 animals of group 2 on the 8th, 15th, and 22nd days of growth of the tumor; partial hepatectomy by the method of Higgins and Anderson was performed on the rats of group 3 on the 8th and 15th days of tumor growth. The animals were decapitated 1, 2, 4, 8, 16, 22, 30, and 45 days after the beginning of the experiment, and the body of the rats and the tumor and liver were weighed. Groups of 10-15 animals were sacrificed 8, 16, and 22 days after the beginning of the experiment, and groups of 5-7 animals at other times. Pieces of liver were fixed in Zenker-formol and in 12% neutral formalin solution and embedded in celloidin-paraffin. Sections were stained with hematoxylin-eosin, glycogen was detected by the PAS-reaction, lipids by Sudan III, and the argyrophilic matrix of the liver was impregnated with silver by Foot's method. The concentration of nuclei was determined by counting them in a standard field of vision (50-60 fields of vision containing 4440-5800 cells were examined). The number of hepatocytes dividing by mitosis and the number of binuclear hepatocytes were counted. The diameter of the nuclei was determined by measuring 100 nuclei with an ocular micrometer in each case. To assess regeneration of the liver its relative weight was obtained and the increase in weight of the regenerating liver expressed as a percentage of the initial weight of the liver residue [6]. The glycogen and lipid content and the size and number of the foci of necrosis were assessed by a 4-point system.

EXPERIMENTAL RESULTS

During growth of the tumor, inflammatory, degenerative, and compensatory-regenerative changes developed in the rats' liver. The absolute weight of the liver in the animals with tumors was significantly

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TABLE 1. Changes in Regenerating Liver of Rats Without Tumors and With Sarcoma M1

Day after operation	Mitosis (in ‰)	Binuclear hepatocytes (in ‰)	Concentration of nuclei	Diameter of nuclei (in μ)	Glycogen	Lipids	Foci of necrosis
			Animals without tumors				
0	0,17±0,006	60±1	145±1	6,72±0,14	+++	0	0
1	49±1,96	50±2	122±1	7,8±0,25	0	++	0
2	22±3	35,±2	126±1	7,28±0,25	+	+	0
4	5,9±1	23±2	137±2	7,1±0,196	+	+	0
8	1,8±0,36	17±1	150±2	7,0±0,28	++	0	0
16	4,1±0,5	26±1	136±1	6,9±0,224	++	0	0
30	0,24±0,004	37±1	142±1	6,8±0,148	+++	0	0
			Partial hepatectomy on 8th day of tumor growth				
0	2,0±0,026	102±3	147±1	7,0±0,14	+	+	+
1	20,0±3,3	43±3	123±2	7,6±0,28	0	++	+
2	38,0±1,9	49±2	122±1	8,12±0,28	+	++	+
4	7,0±1,09	66±3	128±1	7,0±0,196	+	++	+
8	2,4±0,65	64±4	140±2	6,16±0,196	+	++	+
16	1,5±0,03	62±3	150±1	6,16±0,196	0	++	++
			Partial hepatectomy on 15th day of tumor growth				
0	2,1±0,49	104±2	138±1	7,4±0,14	+	++	++
1	1,8±0,7	76±2,7	127±2	6,8±0,148	0	+++	++
2	6,6±1,5	65±1	122±1	7,6±0,196	0	+++	++
4	4,5±0,54	75±2,7	111±1	7,5±0,196	0	+++	++

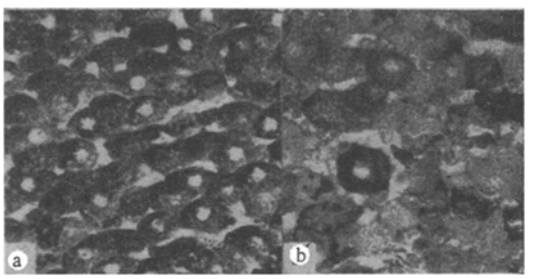

Fig. 1. Changes in liver of rats with sarcoma M1: a) glycogen in liver of normal rat; b) glycogen in liver of rat 22 days after transplantation of sarcoma M1. PAS reaction, 360 ×.

higher than in the control by the 8th day of tumor growth (6.3±0.3 g compared with 5.5±0.22 g), and this difference remained without any significant changes in the future. The relative weight of the liver in the tumor-carrying animals continued to increase throughout the period of observation, and at the end of 1 month it was 5.8±0.3% compared with 3.9±0.1% in the control.

During growth of the subcutaneously transplanted tumors, the glycogen content in the hepatocytes fell progressively (Fig. 1a, b), and fatty degeneration with cloudy swelling and intracellular edema developed in the liver with death of some of the hepatocytes and the formation of foci of necrosis of the parenchyma by the end of 1 month. The compensatory-regenerative changes took the form of an increase in size of the hepatocytes, reflected in a decrease in the concentration of their nuclei in a standard area (147±1 on the 8th day after transplantation of the tumor, 138±1 on the 15th day, and 115±3 on the 22nd day), an increase in the size of the nuclei of the hepatocytes (7.0±0.14 μ on the 8th day after transplantation of the tumor, 7.4±0.14 μ on the 15th day, and 7.8±0.19 μ on the 22nd day), an increase in the number of binuclear hepatocytes (from 22 to 83‰ on the 30th day of tumor growth), and a progressive increase in the number of mitoses (from 1‰ on the 4th day to 12‰ on the 30th day of tumor growth). In the stroma of the liver, coarsening of the argyrophilic matrix and a sharp increase in the number of Kupffer cells were observed.

Healing of the perforating wounds of the liver in the control and experimental rats took place by scar formation. In the parenchyma of the liver of the control animals solitary hepatocytes dividing by mitosis were found near to the wound. In the rats with tumors undergoing operations in the early period of tumor growth the number of mitoses in the hepatocytes around the wound was increased to 1.0±0.07‰, while in those undergoing operation of the 22nd day of growth of the tumor it was 4.0±1.5‰ on the 4th day and 8.0±1.2‰ on the 8th day after wounding, and the nuclei of the hepatocytes were enlarged (7.8±0.28 μ). Healing of the wound in the animals with tumors took place against a background of intensified division of the liver cells, but scar formation was delayed compared with the control. The reason for this was that

the liver tissue of animals with tumors is more sensitive to injury, and after trauma an extensive zone of necrosis is formed, requiring longer time for its organization. A similar picture was observed during healing of skin wounds in rats with transplanted sarcoma 45 [4].

Regeneration of the liver after removal of two-thirds of its mass in the animals with tumors took place in 2 phases. During the first 8 days after partial hepatectomy, restoration of the absolute weight of the liver took place rather more rapidly than in the control (5.3±0.5 and 4.2±0.27 g respectively), while by the 16th day the weight of the regenerating liver was less than in the control (4.6±0.3 and 5.3±0.2 g respectively). The relative weight of the regenerating liver in the animals with tumors was higher than that of the control rats throughout the period of observation.

Mitotic activity of the hepatocytes of the regenerating liver depended on the phase of tumor growth: in rats with small tumors (operation on the 8th day of growth of sarcoma M1) it was below that in the control, and the number of mitoses reached a maximum 48 h, and not 24 h, after the operation (Table 1), although it remained there for a longer time; in the rats with large tumors (operation on the 15th day of growth of the sarcoma) the increase in mitotic activity of the hepatocytes was slight, but the concentration of hepatocyte nuclei was sharply reduced and they were increased in size. In the animals of this series of experiments the tumors grew very rapidly after the operation and the rats died on the 5th-8th day after partial hepatectomy.

The glycogen content in the hepatocytes of the regenerating liver in the animals with tumors was not restored, but by the end of the experiment glycogen had completely disappeared, although in the control its content was fully restored.

Simultaneously with the formation of new hepatocytes in the liver of the animals with tumors, degenerative changes developed which, in some cases, progressed to the formation of foci of necrosis, the number and size of which were greater in the case of partial hepatectomy on the 15th day of tumor growth.

LITERATURE CITED

1. I. F. Prizhivoit and A. I. Sizikov, in: Proceedings of the Second Scientific Conference of Morphologists of Central Asia and Kazakhstan [in Russian], Dushanbe (1968), p. 156.
2. V. G. Rukavishnikova, Changes in the Liver Associated with Primary Cancer in various Locations. Author's abstract of Candidate's Dissertation, Leningrad (1961).
3. L. I. Sergeeva, Disturbances of Liver Function and the Possibility of their Prevention in Patients with Carcinoma of the Esophagus and Carcinoma of the Lungs receiving γ-Ray Teleradiotherapy. Candidate's Dissertation, Frunze (1967).
4. A. I. Sizikov, Byull. Éksperim. Biol. i Med., No. 12, 86 (1965).
5. A. I. Sizikov, in: Problems in Clinical and Experimental Oncology [in Russian], Vol. 3, Frunze (1967), p. 181.
6. G. D. Tumanishvili, Some Problems in the Regulation of Growth of Living Tissues [in Russian], Tbilisi (1965).
7. L. L. Gershbein, J. Nat. Cancer. Inst., 31, 521 (1963).